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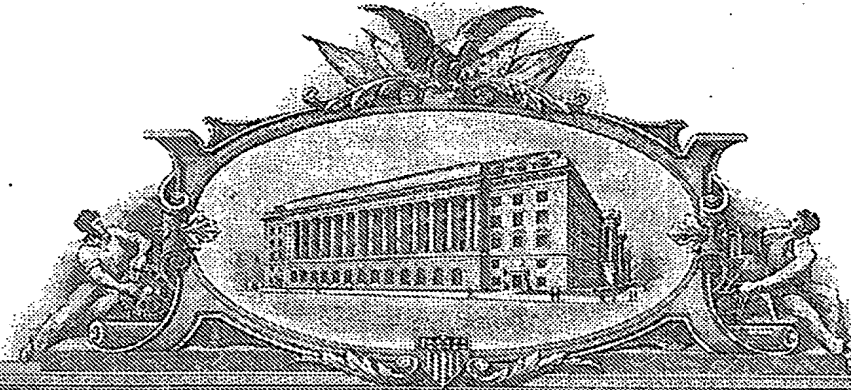
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(b)(2).

INVENTOR(s)/APPLICANT(s)					
Given Name (first and middle [if any])		Family Name or Surname		Residence (CITY AND EITHER STATE OR FOREIGN COUNTRY)	
Allan L.		GOLDSTEIN		Bethesda, Maryland	
<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto.					
TITLE OF THE INVENTION (280 characters max)					
METHOD OF TREATING AND PREVENTING BIOLOGICAL OR IMMUNOLOGICAL RESPONSES TO A REACTIVE CHEMICAL OR BIOLOGICAL OR TOXIC AGENT					
CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number: 6449					
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages [10]		<input type="checkbox"/> CD(s), Number _____			
<input type="checkbox"/> Drawing(s) Number of Sheets []		<input type="checkbox"/> Other (specify) _____			
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27		Filing Fee Amount:			
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fee		\$80.00			
<input type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: <u>02-2135</u>					
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____

Date December 22, 2003

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REGISTRATION NO. 31,414
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USE ONLY FOR FILING PROVISIONAL APPLICATION FOR PATENT



METHOD OF TREATING OR PREVENTING BIOLOGICAL OR IMMUNOLOGICAL RESPONSES TO A REACTIVE CHEMICAL OR BIOLOGICAL OR TOXIC AGENT

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0001] The present invention relates to the field of treating or preventing biological or immunological response to a reactive chemical or biological agent.

DESCRIPTION OF THE BACKGROUND ART

[0002] Contact dermatitis and other allergic reactions due to chemical or biological skin sensitizing agents, environmental toxins or irritants can cause redness, swelling, induration, rashes, blisters, burns, inflammation or eczema skin changes in humans.

[0003] Although many treatments have been proposed for such maladies, there remains a need in the art for improved methods and compositions for treating or preventing the erythema, redness, swelling, induration, rashes, blisters and inflammation due to the physiological and immunological responses to reactive chemicals, biological agents, or toxins.

SUMMARY OF THE INVENTION

[0004] In accordance with the present invention, a method is described for treating or preventing biological or immunological responses to a reactive chemical, biological, or toxic agent. The invention involves administration to a subject or patient in need of such treatment an effective amount of a composition comprising a response-inhibiting polypeptide comprising amino acid sequence LKKTET or a conservative variant thereof having the ability to down-regulate certain inflammatory cytokines and chemokines and other physiological agents and provide relief from the redness, swelling, induration, rashes, blisters, burns or inflammation associated with the host's response to the inducing agent.

DETAILED DESCRIPTION OF THE INVENTION

[0005] The present invention is based on a discovery that actin-sequestering peptides such as thymosin β 4 (T β 4 or TB4) and other actin-sequestering peptides or peptide fragments containing amino acid sequence LKKTET or conservative variants thereof, promote reversal or prevention of a biological or immunological response to a reactive chemical or biological agent. Such biological or immunological responses in a subject may result from exposure to foreign chemical or biological agents, which produce a biological or immunological response in the subject. The invention is applicable to conditions including, but not limited to, the following: dermatologic and other disorders due to allergic reactions, reactions to chemicals and toxins, contact dermatitis, and reactions to plants including, but not limited to, poison ivy, poison oak, and poison sumac; bites of insects including, but not limited to, mosquitoes, fire ants, chiggers, ticks, bees, spiders, fleas and flies; bites of reptiles, especially venomous reptiles, amphibians, and other animals; contact with various animals with venom on their skin such as poisonous frogs; and allergic reactions of the pulmonary and gastrointestinal systems. The invention is also applicable to skin sensitizing agents, psoriasis, atopic dermatitis and eczemas and other conditions that may present with scaling patches and plaques or with bullous and vesicular changes. The invention is also applicable to occupational allergic contact dermatitis, such as but not limited to nickel-associated dermatitis.

[0006] Thymosin β 4 was initially identified as a protein that is up-regulated during endothelial cell migration and differentiation *in vitro*. Thymosin β 4 was originally isolated from the thymus and is a 43 amino acid, 4.9 kDa ubiquitous polypeptide identified in a variety of tissues. Several roles have been ascribed to this protein including a role in a endothelial cell differentiation and migration, T cell differentiation, actin sequestration, vascularization and wound healing.

[0007] In accordance with one embodiment, the invention is a method of treating or preventing biological or immunological responses to a reactive chemical or biological agent comprising administering to a subject in need of such treatment an effective amount of a composition comprising a biological or immunological response-inhibiting peptide comprising amino acid sequence LKKTET, or a conservative variant thereof having biological or immunological response-inhibiting activity,

preferably Thymosin β 4, an isoform of Thymosin β 4, oxidized Thymosin β 4 or an antagonist of Thymosin β 4.

[0008] Compositions which may be used in accordance with the present invention include Thymosin β 4 (T β 4), T β 4 isoforms, oxidized T β 4, polypeptides or peptide fragments comprising or consisting essentially of the amino acid sequence LKKTET or conservative variants thereof, having biological or immunological response-inhibiting activity. International Application Serial No. PCT/US99/17282, incorporated herein by reference, discloses isoforms of T β 4 which may be useful in accordance with the present invention as well as amino acid sequence LKKTET and conservative variants thereof having biological or immunological response-inhibiting activity, which may be utilized with the present invention. International Application Serial No. PCT/GB99/00833 (WO 99/49883), incorporated herein by reference, discloses oxidized Thymosin β 4 which may be utilized in accordance with the present invention. Although the present invention is described primarily hereinafter with respect to T β 4 and T β 4 isoforms, it is to be understood that the following description is intended to be equally applicable to amino acid sequence LKKTET, peptides and fragments comprising or consisting essentially of LKKTET, conservative variants thereof having biological or immunological response-inhibiting activity, as well as oxidized Thymosin β 4.

[0009] In one embodiment, the invention provides a method of treating or preventing biological or immunological responses to a reactive chemical or biological agent in a subject by contacting the skin with a biological or immunological response-inhibiting effective amount of a composition which contains T β 4 or a T β 4 isoform. The contacting may be topically or systemically. Examples of topical administration include, for example, contacting the skin with a lotion, salve, gel, cream, paste, spray, suspension, dispersion, hydrogel, ointment, or oil comprising T β 4. Systemic administration includes, for example, intravenous, intraperitoneal, intramuscular injections of a composition containing T β 4 or a T β 4 isoform. A subject may be a mammal, preferably human.

[0010] T β 4, or its analogues, isoforms or derivatives, may be administered in any suitable biological or immunological response-inhibiting amount. For example, T β 4 may be administered in dosages within the range of about 0.1-50 micrograms of T β 4, more preferably in amounts of about 1-25 micrograms.

[0011] A composition in accordance with the present invention can be administered daily, every other day, etc., with a single application or multiple applications per day of administration, such as applications 2, 3, 4 or more times per day of administration.

[0012] T β 4 isoforms have been identified and have about 70%, or about 75%, or about 80% or more homology to the known amino acid sequence of T β 4. Such isoforms include, for example, T β 4^{ala}, T β 9, T β 10, T β 11, T β 12, T β 13, T β 14 and T β 15. Similar to T β 4, the T β 10 and T β 15 isoforms have been shown to sequester actin. T β 4, T β 10 and T β 15, as well as these other isoforms share an amino acid sequence, LKKTET, that appears to be involved in mediating actin sequestration or binding. Although not wishing to be bound to any particular theory, the activity of T β 4 isoforms may be due, in part, to the ability to polymerize actin. For example, T β 4 can modulate actin polymerization in skin (e.g. β -thymosins appear to depolymerize F-actin by sequestering free G-actin). T β 4's ability to modulate actin polymerization may therefore be due to all, or in part, its ability to bind to or sequester actin via the LKKTET sequence. Thus, as with T β 4, other proteins which bind or sequester actin, or modulate actin polymerization, including T β 4 isoforms having the amino acid sequence LKKTET, are likely to be effective, alone or in a combination with T β 4, as set forth herein.

[0013] Thus, it is specifically contemplated that known T β 4 isoforms, such as T β 4^{ala}, T β 9, T β 10, T β 11, T β 12, T β 13, T β 14 and T β 15, as well as T β 4 isoforms not yet identified, will be useful in the methods of the invention. As such T β 4 isoforms are useful in the methods of the invention, including the methods practiced in a subject. The invention therefore further provides pharmaceutical compositions comprising T β 4, as well as T β 4 isoforms T β 4^{ala}, T β 9, T β 10, T β 11, T β 12, T β 13, T β 14 and T β 15, and a pharmaceutically acceptable carrier.

[0014] In addition, other proteins having actin sequestering or binding capability, or that can mobilize actin or modulate actin polymerization, as demonstrated in an appropriate sequestering, binding, mobilization or polymerization assay, or identified by the presence of an amino acid sequence that mediates actin binding, such as LKKTET, for example, can similarly be employed in the methods of the invention. Such proteins include gelsolin, vitamin D binding protein (DBP), profilin, cofilin, depactin, Dnasel, villin, fragmin, severin, capping protein, β -actinin and acumentin,

for example. As such methods include those practiced in a subject, the invention further provides pharmaceutical compositions comprising gelsolin, vitamin D binding protein (DBP), profilin, cofilin, depactin, DnaseI, vilin, fragmin, severin, capping protein, β -actinin and acumentin as set forth herein. Thus, the invention includes the use of an EB-inhibiting polypeptide comprising the amino acid sequence LKKTET and conservative variants thereof.

[0015] As used herein, the term "conservative variant" or grammatical variations thereof denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the replacement of a hydrophobic residue such as isoleucine, valine, leucine or methionine for another, the replacement of a polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like.

[0016] T β 4 has been localized to a number of tissue and cell types and thus, agents which stimulate the production of T β 4 can be added to or comprise a composition to effect T β 4 production from a tissue and/or a cell. Such agents include members of the family of growth factors, such as insulin-like growth factor (IGF-1), platelet derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor beta (TGF- β), basic fibroblast growth factor (bFGF), thymosin α 1 (T α 1) and vascular endothelial growth factor (VEGF). More preferably, the agent is transforming growth factor beta (TGF- β) or other members of the TGF- β superfamily. T β 4 compositions of the invention may reduce the affects of biological or immunological response to a reactive chemical or biological agent by effectuating growth of the connective tissue through extracellular matrix deposition, cellular migration and vascularization of the skin.

[0017] In accordance with one embodiment, subjects are treated with an agent that stimulates production in the subject of a biological or immunological response-inhibiting peptide as defined above.

[0018] Additionally, agents that assist in reduction of biological or immunological response to a reactive chemical or biological agent may be added to a composition along with T β 4 or a T β 4 isoform. Such agents include angiogenic agents, growth factors, agents that direct differentiation of cells, agents that promote migration of cells and agents that stimulate the provision of extracellular matrix material in the skin. For example, and not by way of limitation, T β 4 or a T β 4 isoform alone or in

combination can be added in combination with any one or more of the following agents: VEGF, KGF, FGF, PDGF, TGF β , IGF-1, IGF-2, IL-1, prothymosin α and thymosin α 1 in an effective amount.

[0019] The invention also includes a pharmaceutical composition comprising a therapeutically effective amount of T β 4 or a T β 4 isoform in a pharmaceutically acceptable carrier. Such carriers include those listed above with reference to parenteral administration.

[0020] The actual dosage or reagent, formulation or composition that reverses or prevents biological or immunological response to a reactive chemical or biological agent may depend on many factors, including the size and health of a subject. However, persons of ordinary skill in the art can use teachings describing the methods and techniques for determining clinical dosages as disclosed in PCT/US99/17282, *supra*, and the references cited therein, to determine the appropriate dosage to use.

[0021] Suitable topical formulations include T β 4 or a T β 4 isoform at a concentration within the range of about 0.001 - 10% by weight, more preferably within the range of about 0.01 - 0.1% by weight, most preferably about 0.05% by weight.

[0022] The therapeutic approaches described herein involve various routes of administration or delivery of reagents or compositions comprising the T β 4 or other compounds of the invention, including any conventional administration techniques (for example, but not limited to, topical administration, local injection, inhalation, or systemic administration), to a subject. The methods and compositions using or containing T β 4 or other compounds of the invention may be formulated into pharmaceutical compositions by admixture with pharmaceutically acceptable non-toxic excipients or carriers.

[0023] The invention includes use of antibodies which interact with T β 4 peptide or functional fragments thereof. Antibodies which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art as disclosed in PCT/US99/17282, *supra*. The term antibody as used in this invention is meant to include monoclonal and polyclonal antibodies.

[0024] In yet another embodiment, the invention provides a method of treating a subject by administering an effective amount of an agent which modulates T β 4 gene expression. The term "modulate" refers to inhibition or suppression of T β 4 expression when T β 4 is over expressed, and induction of expression when T β 4 is underexpressed. The term "effective amount" means that amount of T β 4 agent which is effective in modulating T β 4 gene expression resulting in reducing the symptoms of the biological or immunological response to a reactive chemical or biological agent. An agent which modulates T β 4 or T β 4 isoform gene expression may be a polynucleotide for example. The polynucleotide may be an antisense, a triplex agent, or a ribozyme. For example, an antisense directed to the structural gene region or to the promoter region of T β 4 may be utilized. The agent which modulates T β 4 or T β 4 isoform gene expression may also be a small interfering RNAs (siRNAs).

[0025] In another embodiment, the invention provides a method for utilizing compounds that modulate T β 4 activity. Compounds that affect T β 4 activity (e.g., antagonists and agonists) include peptides, peptidomimetics, polypeptides, chemical compounds, minerals such as zincs, and biological agents.

Example

[0026] One area of skin surface with a visible redness, induration, swelling and erythema due to exposure to poison ivy was treated by topical application of a medicament containing 2 % by weight T β 4, while another area of visible redness, induration, swelling and erythema due to exposure to poison ivy reaction on the same skin surface was left untreated. After one day, induration and erythema in the treated area were significantly reduced as compared to the untreated area, and itching of the treated area was significantly less than the untreated area.

CLAIMS

1. A method of treating or preventing biological or immunological responses such as redness, induration, swelling, itching and erythema following exposure to a reactive chemical or biological agent or toxin, comprising administering to a subject in need of such treatment an effective amount of a composition comprising a response-inhibiting polypeptide comprising amino acid sequence LKKTET, or a conservative variant thereof having the ability to down-regulate specific inflammatory cytokines and chemokines and thereby result in biological or immunological response-inhibiting activity.
2. The method of claim 1 wherein said polypeptide comprises Thymosin β 4 (T β 4), an isoform of T β 4 or oxidized T β 4.
3. The method of claim 1 wherein said composition is administered systemically.
4. The method of claim 1 wherein said composition is administered topically.
5. The method of claim 4 wherein said composition is in the form of a gel, creme, paste, lotion, spray, suspension, dispersion, salve, hydrogel or ointment formulation.
6. The method of claim 1 wherein said polypeptide is recombinant or synthetic.
7. The method of claim 1 wherein said polypeptide is an antibody.
8. The method of claim 7 wherein said antibody is polyclonal or monoclonal.
9. A method of treating or preventing biological or immunological responses to a reactive chemical or biological agent comprising administering to a subject in need of such treatment an effective amount of a composition comprising an agent

that stimulates production of a biological or immunological response-inhibiting polypeptide comprising amino acid sequence LKKTET, or a conservative variant thereof having biological or immunological response-inhibiting activity.

10. The method of claim 9 wherein said polypeptide is Thymosin β 4.

11. The method of claim 9 wherein said agent is an antagonist of Thymosin β 4.

12. A composition for use in promoting reversal or prevention of biological or immunological response to a reactive chemical or biological agent comprising an effective amount of a composition including a biological or immunological response-inhibiting polypeptide comprising amino acid sequence LKKTET or a conservative variant thereof having biological or immunological response-inhibiting activity.

13. The composition of claim 12 wherein said polypeptide comprises T β 4, an isoform of T β 4 or oxidized T β 4.

14. The composition of claim 12, comprising a gel, creme, paste, lotion, spray, suspension, dispersion salve, hydrogel or ointment formulation.

15. Therapeutic method for treating an inflammatory skin or mucosal membrane condition due to an environmental toxin or skin-sensitizing agent in a mammal in need of such therapy by administering topically, systemically and effective amount of TB4 or an isoform, or splice variant, or fragment, of TB4 in a suitable pharmaceutical composition.

ABSTRACT OF THE DISCLOSURE

[0027] Biological or immunological responses to a reactive chemical or biological agent erythematic skin rashes with redness or swelling or induration, blistering and itching is treated or prevented by administration of an actin-sequestering peptide such as Thymosin β 4, an isoform or splice variant of Thymosin β 4 or oxidized Thymosin β 4 and a number of other compounds containing the amino sequence LKKTET.

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